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Identification of PPARγ agonists in root extracts of purple coneflower (*Echinacea purpurea*) by bioassay-guided fractionation

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Introduction

One of the major characteristics of type 2 diabetes (T2D) is insulin resistance, which is often treated by insulin sensitizing drugs such as thiazolidinediones (TZDs). The primary target for the TZDs is the peroxisome proliferator-activated receptor PPARγ. However, critical side effects of TZDs can occur, as they are full PPARγ agonists. Partial PPARγ agonists are associated with fewer side effects but still may maintain the effect on insulin resistance. Alkamides are very similar in chemical structure to natural ligands for the PPARγ such as fatty acids, and hence are potential PPARγ agonists. Recently, a new C_{16}-alkamide able to activate PPARγ with no concurrent stimulation of adipocyte differentiation, and able to increase insulin-stimulated glucose uptake was isolated from the flowers of purple coneflower (*Echinacea purpurea*) [1]. In our search for new partial PPARγ agonists the roots of purple coneflower, which is a rich source for alkamides, was investigated.

Extraction and isolation

5 kg fresh roots of purple coneflower were extracted overnight with dichloromethane (DCM). The dried extract was subjected to bioassay-guided chromatographic fractionation. First step was a separation by flash column chromatography using a *n*-hexane–ethyl acetate gradient resulting in 10 fractions (A–J). Two fractions (D and H) showed promising bioactivity in a dose-dependent manner. LC-MS analysis revealed that these two fractions primarily contained known and unknown alkamides (data not shown).

PPARγ transactivation assay

The ability of the DCM extract to activate the PPARγ was tested in a luciferase-based PPAR transactivation assay using mouse embryonic fibroblasts transfected with a luciferase reporter plasmid, a transfection control plasmid, and an expression plasmid. Degree of activation was determined by a luminometer and compared to a positive control, Rosiglitazone (Rosi). The DCM extract was found to activate PPARγ in a dose-dependent manner (1–100 µg/mL) without stimulating adipocyte differentiation at 100 µg/mL. Figures 1 and 2 show fold activations of PPARγ by the DCM extract with vehicle (DMSO) set to 1.0 and corresponding results from adipocyte differentiation assay (DEX protocol).

Fig. 1

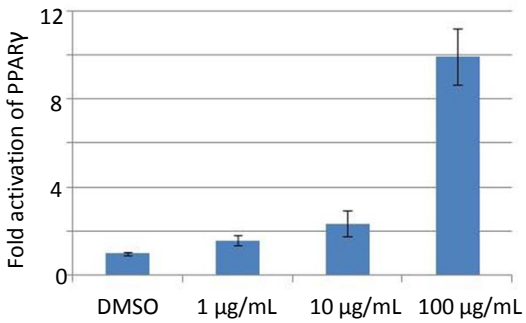
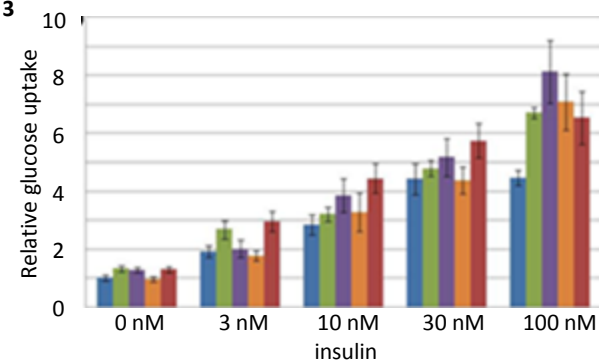


Fig. 2

Stimulation of glucose uptake in adipocytes

The DCM extract and fractions D and H were tested for stimulation of insulin-dependent glucose uptake in adipocytes. Dose-dependent stimulation (0–100 nM) was observed for all three samples as shown in Figure 3. All results were compared to a positive control (Rosi) and vehicle (DMSO). Mature adipocytes were subjected to the extract/fractions and after 2 days glucose uptake was induced by insulin. Effect was measured using ^{14}C-labeled glucose and afterwards radioactivity was determined by scintillation counting.

Fig. 3



Conclusions and Perspectives

- The DCM extract of the roots of purple coneflower showed promising activation of PPARγ in a dose-dependent manner without stimulating adipocyte differentiation.
- Bioassay-guided fractionation resulted in two alkamide-rich fractions, which were able to activate PPARγ and positively affected insulin-stimulated glucose uptake in adipocytes.
- The results of this study clearly indicate that alkamides are partial PPARγ agonists with a potential in relation to the management of insulin resistance and T2D. Individual alkamides from the active fractions warrant further investigation for their PPARγ activating properties in order to identify the most active alkamides and hence, the most promising PPARγ agonists in coneflower roots.

Reference

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